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RP-HPLC Method Development and Validation of Indapamide And Perindopril In Pharmaceutical Dosage Forms

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ABSTRACT

A simple, precise and rapid HPLC method has been developed for the simultaneous determination of Indapamide and Perindopril in pharmaceutical dosage form. The method was carried out using Hypersil BDS C18 column (250 mm x 4.6 mm, 5 μ m) and mobile phase of phosphate buffer pH 3.5 \pm 0.05 and methanol in the ratio of 65:35 v/v and degassed under ultrasonication. The flow rate was 1.0 mL/min and the effluent was monitored at 215 nm. The retention times of Perindopril and Indapamide were 3.53 min and 4.09 min respectively. The method was validated. Linearity was in the range of 160 to 480 μ g/mL for Perindopril and 50 to 150 μ g/mL for Indapamide respectively. The percentage recoveries of both the drugs were ranging from 97.8 to 101.7 for Perindopril and 98.7 to 101.8 for Indapamide respectively from the tablet formulation. The proposed method is suitable for the routine quality control analysis of simultaneous determination of Perindopril and Indapamide in pharmaceutical dosage form.

Keywords: Perindopril, Indapamide, RP-HPLC, C18 (4.6*250) mm, 5 micron column, Validation. Perindopril; Indapamide; RP-HPLC; Validation.

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INTRODUCTION

Perindopril erbumine (Figure 1) is a pro-drug for perindoprilat, non-sulfhydryl converting enzyme (ACE) inhibitor ¹. Chemically it is butylalanylhexahydro-2-indoline carboxylic acid, 1-ethyl ester, compound with tert-butylamine (1:1) ². The mechanism through which perindoprilat lowers blood pressure is believed to be primarily inhibition of ACE activity ³. Perindopril erbumine is converted to Perindoprilat in the liver and is used to treat hypertension and heart failure, to reduce proteinuria and renal disease in patients with nephropathies, and to prevent stroke, myocardial infarction, and cardiac death in highrisk patients. It may be used alone or in combination with other antihypertensive agents. buffered by Perindopril due to its potassium-sparing effect ⁷. Indapamide (Figure 2) is a nonthiazide indole derivative of chlorosulfonamide ⁴. Indapamide is used in management of mild to moderate hypertension as an oral antihypertensive/diuretic, treatment of edema in congestive heart failure and nephrotic syndrome ⁵⁻⁶. Chemically it is 4- chloro-N-(2-methyl-2,3-dihydro-1H-indol-1-yl)-3- sulfamoyl benzamide. Indapamide causes the blood vessels to widen, which reduces the pressure inside the blood vessels. This helps to lower blood pressure. The combination of Perindopril and Indapamide is recommended due to their synergistic mechanisms of action. On the one hand, as Indapamide depletes the cell of sodium and of calcium, this reduces the vascular response to angiotensin II and on the other hand, Perindopril blocks the activation of RAAS and sympathetic nervous system induced by Indapamide. Moreover, the potassium depletion caused by Indapamide. Literature survey reveals that few analytical methods have been reported for Perindopril and Indapamide individually in biological fluids and in pharmaceutical dosage forms. Few analytical methods using spectrophotometry ⁸⁻⁹, HPLC ¹⁰⁻¹³ and HPTLC ¹⁴ have been reported for the simultaneous determination of Perindopril and Indapamide in combined dosage forms. The objective of the present study was to develop and validate a simple, accurate and precise HPLC method for simultaneous determination of Perindopril and Indapamide.

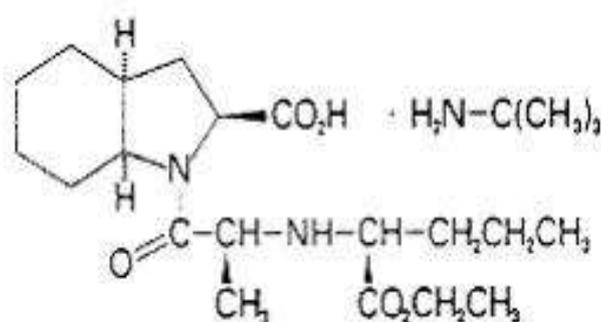


Figure 1: Chemical structure of Perindopril erbumine

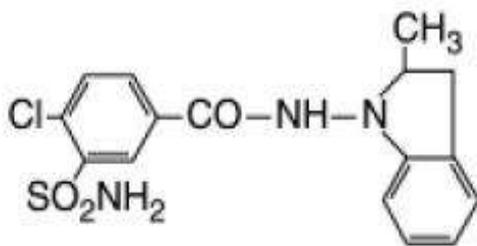


Figure 2: Chemical structure of Indapamide

MATERIALS AND METHOD

Instrumentation:

The analysis of drugs was carried out on a Waters HPLC system on a Hypersil BDS C18 column (250 mm x 4.6 mm, 5 μ m). The instrument is equipped with a LC20-AT with photodiode array detector (PDA) and a Rheodyne injector with 20 μ L sample loop. A 20 μ L syringe was used for injecting the samples. A double-beam Shimadzu UV-Visible 1700 spectrophotometer was used for spectral studies. Degassing of the mobile phase was done by using an ultrasonic bath sonicator. A Shimadzu balance was used for weighing the materials.

Chemicals and Solvents:

The reference samples of Perindopril erbumine and Indapamide were obtained from Serdia Pharmaceuticals, Mumbai, India. The branded formulations (tablets) (Coversyl-Plus) tablets containing 4 mg of Perindopril and 1.25 mg of Indapamide were procured from the local market. HPLC grade methanol and analytical grade potassium dihydrogen phosphate, orthophosphoric acid was obtained from Qualigens Fine Chemicals Ltd, Mumbai, India. Hydrochloric acid, sodium hydroxide, hydrogen peroxide of analytical grade was obtained from Merck Chemicals Ltd, Mumbai, India. Milli-Q water was used throughout the experiment dispensed through 0.22 μ filter of the Milli-Q water purification system from Millipore, Merck, Germany.

Chromatographic conditions:

HPLC was connected with Hypersil BDS C18 column (250 mm x 4.6 mm, 5 μ m) as stationary phase. A mixture of phosphate buffer pH 3.5 \pm 0.05 and methanol in the ratio of 65:35 v/v was prepared and used as mobile phase. The phosphate buffer solution was prepared by weighing about 6.8 grams of potassium dihydrogen phosphate and transfer to 1000 mL standard flask, add 400 mL of Milli-Q water, mix and dilute to volume with Milli-Q water, sonicate for five minutes and cool to room temperature, measure the pH of above buffer solution and finally adjusted the pH to 3.5 with orthophosphoric acid solution and filtered through 0.45 μ nylon filter. The mixture of water and methanol in the ratio of 80:20 v/v was prepared and used as diluent. Injection volume was 5 μ L and flow rate was 1.0 mL/min and run time was 7.0 min. The column was maintained at ambient temperature and

the eluent was monitored at 215 nm.

Preparation of standard solution:

About 80 mg of Perindopril and 25 mg of Indapamide were accurately weighed and transferred into a 50 mL clean dry volumetric flask containing 25 mL of diluent and sonicated for 30 min. The solution were cooled to room temperature and diluted to volume with diluent and used as standard stock solution. Standard stock solution was diluted to get a concentration of 160-480 $\mu\text{g/mL}$ and 50-150 $\mu\text{g/mL}$ for Perindopril and Indapamide respectively.

Preparation of sample solution:

Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 80 mg of Perindopril and 25 mg of Indapamide was transferred to a 50 mL volumetric flask containing 25 mL of the diluent. The contents of the flask were sonicated for about 30 min for complete solubility of the drug and volume made up with further quantity of diluent. Then this mixture was filtered through 0.45 μ membrane filter. 5.0 mL of this filtrate was further diluted to 25 mL with diluent.

Method development:

To develop a simple and robust method for the simultaneous determination of Perindopril and Indapamide in combined tablet dosage form using HPLC. The spectra of diluted solutions of the Perindopril erbumine and Indapamide in methanol were recorded separately on UV spectrophotometer. The peaks of maximum absorbance wavelengths were observed. The spectra of the both Perindopril erbumine and Indapamide were showed that a balanced wavelength was found to be 215 nm. Preliminary development trials have performed with octyl and octadecyl columns with different types, configurations and from different manufacturers. Finally the expected separation and shapes of peak was succeeded in Hypersil BDS C18 column.

To effect ideal separation of the drug under isocratic conditions, mixtures of solvents like water, methanol and acetonitrile with or without different buffers in different combinations were tested as mobile phases on a C18 stationary phase. A mixture of phosphate buffer pH 3.5 and methanol in proportion of ratio 65:35 v/v was proved to be the most suitable of all the combinations since the chromatographic peaks obtained were better defined and resolved and almost free from tailing. Flow rates of the mobile phase were changed from 0.5-2.0 mL/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found from the experiments that 1.0 mL/min flow rate was ideal for the successful elution of the analyte. No interference in blank and placebo solutions for both drug peaks in the trail injections with a runtime of 7.0 min.

The above optimized chromatographic conditions were followed for the simultaneous determination of Perindopril erbumine and Indapamide in bulk samples and its combined tablet formulations. The chromatograms of standard and sample solutions of Perindopril erbumine and Indapamide were shown in Figure 3 and Figure 4.

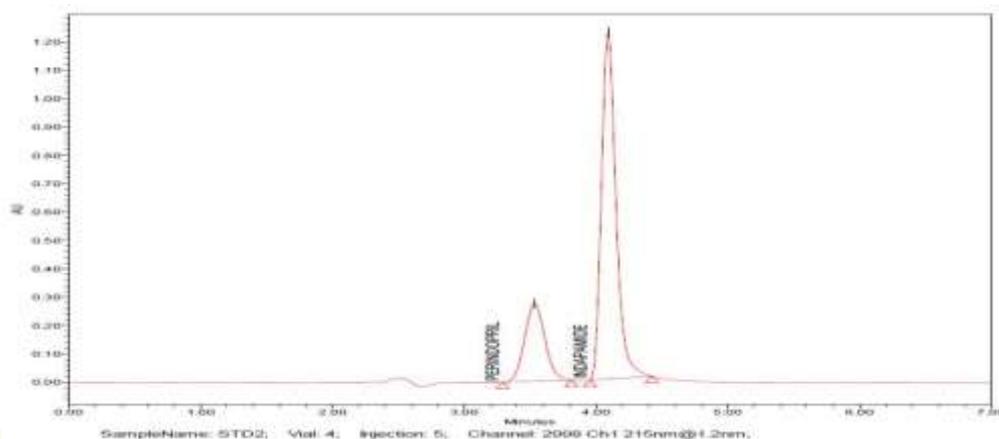


Figure 3: Chromatogram of standard solution of Perindopril and Indapamide

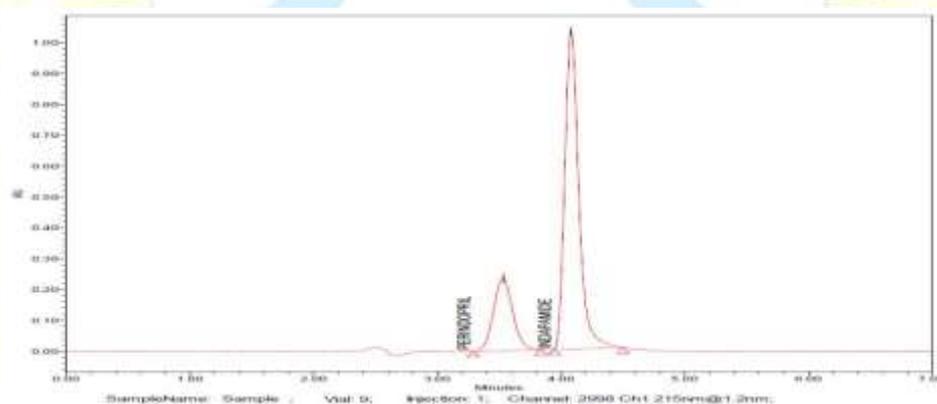


Figure 4: Chromatogram of sample solution of Perindopril and Indapamide

Validation of the proposed method:

The proposed method was validated as per ICH¹⁵ guidelines. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and solution stability.

Specificity:

A study conducted to establish specificity of the proposed method involved injecting blank and placebo using the chromatographic conditions defined for the proposed method. It was found that there is no interference due to excipients in the tablet formulation and also found good correlation between the retention times of standard and sample. **Linearity:**

Linearity was performed by preparing mixed standard solutions of Perindopril and Indapamide at different concentration levels including working concentration mentioned in experimental condition i.e., 160 to 480 µg/mL for Perindopril and 50 to 150 µg/mL for Indapamide respectively. Five microlitres of each concentration was injected in duplicate into

the HPLC system. The response was read at 215 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were calculated and linearity plots of concentration over the mean peak areas were constructed individually.

The regressions of the plots were computed by least square regression method. Linearity results were presented in Table 1 & 2 and linearity plots are shown in Fig. 5 & 6. In linearity, working concentration mentioned in experimental condition i.e., 1599.64 $\mu\text{g/mL}$ for Perindopril erbumine and 502.60 $\mu\text{g/mL}$ for Indapamide respectively.

Table 1: Linearity study of Perindopril

Level	Concentration of Perindopril ($\mu\text{g/mL}$)	Mean peak area
Level-1	159.96	1461519
Level-2	239.95	2206262
Level-3	319.93	2933885
Level-4	391.91	3664520
Level-5	479.89	4402238
Slope		9263.0
Intercept		-15043.0
Correlation Coefficient		0.9997
Residual Sum of Squares		33918.0

Range: 50 to 150 % of target concentration (i.e. 160 to 480 $\mu\text{g/mL}$)

Table 2: Linearity study of Indapamide

Level	Concentration of Indapamide ($\mu\text{g/mL}$)	Mean peak area
Level-1	50.26	4676540
Level-2	75.39	7028273
Level-3	100.52	9379571
Level-4	123.14	11740253
Level-5	150.78	14087045
Slope		94522.0
Intercept		-71461.0
Correlation Coefficient		0.9996
Residual Sum of Squares		117972.0

Precision:

Precision is the degree of repeatability of an analytical method under normal operational conditions. Precision of the method was performed as system precision, method precision and intermediate precision.

System precision:

To study the system precision, five replicate mixed standard solutions of Perindopril and Indapamide were injected. The percent relative standard deviation (% RSD) was calculated and it was found to be 1.2 and 1.0 for Perindopril and Indapamide respectively, which are well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in Table 3.

Table 3: System precision

Injection number	Area of Perindopril	Area of Indapamide	Acceptance criteria
1	2857397	9160424	The %RSD Of peak areas of Perindopril And Indapamide should Not be more than 2.0
2	2917302	9332438	
3	2932729	9397414	
4	2941434	9350222	
5	2946364	9378691	
Mean	2919045	9323838	
%RSD	1.2	1.0	

Method precision:

The method precision study was carried out on six preparations from the same tablet samples of for Perindopril and Indapamide and percent amount of both were calculated. The %RSD of the assay result of six preparations in method precision study was found to be 0.7 and 0.4 for Perindopril and Indapamide respectively, which are well within the acceptance criteria of not more than 2.0. The results obtained for assay of for Perindopril and Indapamide are presented in Table 4.

Table 4: Method precision

Sample number	% Assay	
	Perindopril	Indapamide
1		98.7
2		99.6
3		98.8
4		99.1
5		99.3
6		98.9
Mean	99.0	99.0
%RSD	0.7	0.4

Intermediate precision:

The intermediate precision study was carried out by different analysts, different columns, different reagents using different HPLC systems from the same tablet of for Perindopril and Indapamide and the percent amount of for Perindopril and Indapamide was calculated. The %RSD of the assay result of six preparations in intermediate precision study was 0.2 and 0.5 for Perindopril and Indapamide respectively, which are well within the acceptance criteria of not more than 2.0. The results of intermediate precision study are reported in Table 5 & 6.

Table 5: Intermediate precision study of Perindopril

Preparation number	% Assay	Mean	%RSD
1	99.1	99.0	0.2
2	98.9		
3	99.3		
4	99.1		
5	98.7		
6	98.8		

Table 6: Intermediate precision study of Indapamide

Preparation number	% Assay	Mean	%RSD
1	100.9		
2	100.6		
3	101.7		
4	101.2		
5	100.2		
6	100.8	100.9	0.5

Accuracy:

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. The standard addition method was performed at three concentration levels of 50%, 100% and 150%. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and %RSD at each level was calculated and results are presented in Table 7 & 8. Satisfactory recoveries ranging from 97.8 to 101.7 for Perindopril and 98.7 to 101.8 for Indapamide respectively were obtained by the proposed method. This indicates that the proposed method was accurate.

Table 7: Recovery study for Perindopril

Level	Amount of Indapamide spiked	Amount of Indapamide recovered	% Recovery	%RSD
50%	158.33	157.43	99.4	1.1
	158.33	157.66	99.4	
	158.45	155.04	97.8	
	157.36	154.84	98.4	
	158.20	158.40	100.1	
100%	158.72	160.05	100.8	1.3
	316.84	314.26	99.2	
	316.93	319.19	100.7	
150%	317.00	322.44	101.7	0.4
	475.13	476.40	100.3	
	474.95	476.48	100.3	
	475.65	477.91	100.5	
	474.13	478.51	100.9	
	475.33	478.93	100.8	
	474.68	481.34	101.4	
Mean % recovery				100.1
Overall %RSD				1.1

Table 8: Recovery study for Indapamide

Level	Amount of Indapamide spiked	Amount of Indapamide recovered	% Recovery	%RSD
50%	50.43	50.11	99.4	0.4
	50.50	50.04	99.1	
	50.47	49.83	98.7	
	50.12	50.04	99.8	

	50.39	50.10	99.4	
	50.55	50.27	99.4	
100%	100.91	100.67	99.8	0.3
	100.94	100.66	99.7	
	100.96	100.14	99.2	
150%	151.33	150.82	99.7	
	151.27	150.89	99.7	
	151.49	152.03	100.4	0.8
	151.01	153.74	101.8	
	151.39	151.81	100.3	
	151.18	151.57	100.3	
Mean % recovery				99.8
Overall %RSD				0.7

Robustness:

The robustness study was performed by slight modification in flow rate of the mobile phase, pH of the buffer and composition of the mobile phase. The samples of Perindopril at 320 µg/mL and Indapamide at 100 µg/mL concentration were analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature.

System suitability:

System suitability was studied under each validation parameters by injecting six replicates of the standard solution. The system suitability parameters are given in Table 9 & 10.

Table 9: System suitability for Perindopril

Parameter	Tailing factor	Theoretical plates
Specificity study	1.03	2560
Linearity study	1.15	2554
Precision study	1.06	2546
Robustness study		
Flow rate at 0.8 mL/min	1.15	2640
Flow rate at 1.2 mL/min	1.12	2830
pH of buffer 3.0	1.08	2340
pH of buffer 4.0	1.14	2435
Mobile phase:		
Buffer(60):Acetonitrile(40)	1.06	2950
Buffer(70):Acetonitrile(30)	1.05	2645

Table 10: System suitability for Indapamide

Parameter	Tailing factor	Theoretical plates
Specificity study	1.33	7033
Linearity study	1.34	7025
Precision study	1.31	7038
Robustness study		
Flow rate at 0.8 mL/min	1.32	6540
Flow rate at 1.2 mL/min	1.29	6425
pH of buffer 3.0	1.27	6652
pH of buffer 4.0	1.36	6520
Mobile phase:		

Buffer(60):Acetonitrile(40)	1.32	7020
Buffer(70):Acetonitrile(30)	1.22	6845

Limit of detection and Limit of quantification:

Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a detectable response. Limit of quantification (LOQ) is defined as the lowest concentration that can be quantified reliably with a specified level of accuracy and precision. For this study six replicates of the analyte at lowest concentration were measured and quantified. The LOD and LOQ of Perindopril and Indapamide are given in Table 11 & 12.

Table 11: LOD and LOQ of Perindopril

Parameter	Measured value (µg/mL)
Limit of detection	12.08
Limit of quantification	36.62

Table 12: LOD and LOQ of Indapamide

Parameter	Measured value (µg/mL)
Limit of detection	4.12
Limit of quantification	12.48

RESULTS AND DISCUSSION

The present study was aimed at developing a simple, sensitive, precise and accurate HPLC method for the simultaneous estimation of Perindopril and Indapamide from bulk samples and their tablet dosage forms. A non-polar C18 analytical chromatographic column was chosen as the stationary phase for the separation and simultaneous determination of Perindopril and Indapamide. Mixtures of commonly used solvents like water, methanol and acetonitrile with or without buffers in different combinations were tested as mobile phases. The choice of the optimum composition is based on the chromatographic response factor, a good peak shape with minimum tailing. A mixture of phosphate buffer pH 3.5±0.05 and methanol in the ratio of 65:35 v/v was proved to be the most suitable of all the combinations since the chromatographic peak obtained was well defined, better resolved and almost free from tailing. The retention times of the Perindopril and Indapamide were found to be 3.53 and 4.09 min respectively.

The linearity was found satisfactory for both the drugs in the range of 160 to 480 µg/mL for Perindopril and 50 to 150 µg/mL for Indapamide respectively. The regression equation of the linearity curve between concentrations of Perindopril and Indapamide over its peak areas were found to be $Y=9263X-15043$ (where Y is the peak area and X is the concentration of Perindopril in µg/mL) and $Y=94522X-71461$ (where Y is the peak area and X is the concentration of Indapamide in µg/mL) respectively. Precision of the method was studied by repeated injection of tablet solution and results showed lower %RSD values. This reveals that

the method is quite precise. The percent recoveries of the drug solutions were studied at three different concentration levels. The percent individual recovery and the %RSD at each level were within the acceptable limits. This indicates that the method is accurate. The absence of additional peaks in the chromatogram indicates non-interference of the commonly used excipients in the tablets and hence the method is specific.

The deliberate changes in the method have not much affected the peak tailing, theoretical plates and the percent assay. This indicates that the present method is robust. The system suitability studies were carried out to check various parameters such as theoretical plates and tailing factor. The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method is sensitive. The solution stability studies indicate that both the drugs were stable up to 24 hours. The forced degradation studies indicate that both the drugs Perindopril and Indapamide were stable in stability studies.

CONCLUSION

The proposed RP-HPLC method was simple, rapid, sensitive, accurate and precise and can be used for simultaneous determination of Perindopril and Indapamide in bulk and its tablet dosage forms.

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REFERENCES

1. The Merck Index, 14th Edn., Merck & Co., Inc., Whitehouse Station, NJ, USA, 2006; 1238.
2. The British Pharmacopoeia Vol. I. The Department of Health, The stationary Office on Behalf of the Medicines and Health Care products Regulatory Agency, London, 2010; 158-159.
3. Bounhoure JP, Bottineau G, Lechat P. Value of Perindopril in the treatment of chronic congestive heart failure: multicentre doubleblind placebo-controlled study. Clin Exp Hypertens, 1989; A11(2): 575-586 .
4. Indian Pharmacopoeia, Govt. of India, Ministry of Health & Family Welfare, The Indian Pharmacopoeia Commission, Ghaziabad, 2010; 2: 1489.
5. CIMS (Current Index of Medical Specialities), UBM Medica India Pvt. Limited,

Bangalore, 2012; October: 111.

6. IDR Drug Triple i Compendium (Indian Drug Review), UBM Medica India Pvt. Limited, Bangalore, 2012; 5: 122.
7. Anushka P. Effects of a fixed combination of Perindopril and Indapamide on macrovascular and microvascular outcomes in patients with type 2 diabetes mellitus (the advance trial): a randomised controlled trial. *Lancet*, 2007; 370: 829-840.
8. Erk N. Comparison of spectrophotometric and an LC method for the determination Perindopril and Indapamide in pharmaceutical formulations. *J Pharm Biomed Anal*, 2001; 26(1): 43-52.
9. Darshana MK, Chhagan PN. Development and validation of spectrophotometric method for simultaneous estimation of Perindopril and Indapamide in combined dosage form by absorbance correction method. *Int J Pharm Tech Res*, 2010; 2(1): 411-416.
10. Jogia H, Khandelwal U, Gandhi T, Singh S. Development and validation of stability-indicating assay method for simultaneous determination of Perindopril and Indapamide in combined dosage form by reversed-phase high-performance liquid chromatography. *J AOAC Int*, 2010; 93(1): 108-115.
11. Pathak AK, Rachana L, Mukesh SK. A selective reverse phase HPLC method for simultaneous determination of Perindopril and Indapamide in solid dosage form and bulk drug. *J Pharm Res*, 2011; 4(8): 2512-2513.
12. Joseph J, Philip B, Sundarapandian M. Method development and validation for simultaneous estimation of Perindopril erbumine and Indapamide by RP-HPLC in pharmaceutical dosage forms. *Int J Pharm Pharm Sci*, 2011; 3(4): 288-293.
13. Rajesh T, Anurekha J, Deepika M, Toppo E. Multicriteria optimization methodology in development of HPLC method for simultaneous estimation of Indapamide and Perindopril in bulk drug and its combined dosage form. *Asian J Pharm Clin Res*, 2012; 5(2): 50-53.
14. Mohit DG, Kailash BG, Ashwini MR, Mrinalini DC. Simultaneous estimation of Perindopril erbumine and Indapamide in Bulk Drug and Tablet Dosage Form by HPTLC. *Int J Comprehensive Pharm*, 2011; 2(1): 1-4.
15. ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology, Q2(R1), USA, 2005; 1-13.

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